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THE OXIDATION OF LINOLEATE AND  
OTHER LONG-CHAIN FATTY ACIDS  
IN RAT AND SHEEP LIVER  
MITOCHONDRIA

A thesis presented in partial fulfilment  
of the requirement for the degree of  
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at  
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John Campbell William Reid  
1986

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### Abstract

Sheep liver mitochondria oxidised palmitate, oleate and linoleate at slower rates than did rat liver mitochondria. Rat liver mitochondria oxidised linoleate at 1.2 to 1.7 times the rate observed with palmitate as the substrate. However, sheep liver mitochondria oxidised linoleate at 0.74 to 0.84 the rate observed when palmitate was the substrate. The biochemical basis of this difference is not understood.

The reaction catalysed by the enzyme carnitine acyltransferase I is believed to be an important regulatory step in the oxidation of long-chain fatty acids and is known to be competitively inhibited by malonyl-CoA. Both rat and sheep liver mitochondria were able to form acyl carnitine when palmitoyl-CoA and linoleate, coupled with an acyl-CoA generating system, were the acyl substrates.

Malonyl-CoA was very effective in inhibiting the CAT I reaction in sheep liver mitochondria. When linoleate, coupled with an acyl-CoA generating system, was the substrate for CAT I, 1  $\mu$ M malonyl-CoA was found to inhibit the reaction by 90%. However, when the same substrate was assayed in rat liver mitochondria the inhibition was much less, 22  $\mu$ M malonyl-CoA leading to only 50% inhibition of the CAT I enzyme. When palmitoyl-CoA was used as a substrate for the enzyme CAT I, little difference was seen between rat and sheep liver mitochondria in the extent of inhibition observed over the concentration range of 1 to 5  $\mu$ M malonyl-CoA.

These experiments indicate that sheep liver mitochondria could oxidise palmitate rather than linoleate at low levels of malonyl-CoA, as one might expect in vivo. In contrast, in rat liver mitochondria, linoleate would be oxidised faster than palmitate at all concentrations of malonyl-CoA investigated.

It is suggested that this system may be an important means whereby sheep are able to conserve linoleate by preventing its oxidation.

In addition the mitochondrial glycerol 3-phosphate acyltransferase reaction was investigated with both sheep and rat liver mitochondria. With linoleate and an acyl-CoA generating system, rat liver preparations esterified 1.5 nmoles min/mg protein whereas sheep liver mitochondria esterified less than one tenth of this. It was concluded esterification of linoleate to glycerol 3-phosphate is not an important mechanism of conserving linoleate in sheep liver mitochondria.

Esterification of palmitate to glycerol 3-phosphate was studied using palmitoyl-CoA as the acyl donor. At maximal rates of esterification it was observed that rat liver mitochondria esterified palmitoyl-CoA at 2 nmoles/min/mg whereas sheep mitochondria esterified 0.8 nmoles/min/mg.

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Abbreviations

|                  |   |  |
|------------------|---|--|
| CAT              | - | carnitine acyltransferase  |
| CPT              | - | carnitine palmitoyltransferase   |
| GPAT             | - | glycerol 3-phosphate acyltransferase                                       |
| FFA              | - | free fatty acid  |
| DTT              | - | dithiothreitol   |
| PPO              | - | 2,5-diphenyloxazole  |
| POPOP            | - | 1,4-bis[2-(5-phenyloxazolyl)]benzene                                       |
| NbS <sub>2</sub> | - | 5,5'-dithiobis-(2-nitrobenzoic acid)                                       |
| EGTA             | - | ethyleneglycol-bis-( $\beta$ -amino-ethyl ether)<br>N,N'-tetra-acetic acid |
| g                | - | gravitational force  |
| g                | - | grams  |
| l                | - | litre  |
| 16:0             | - | palmitic acid  |
| 18:1             | - | oleic acid   |
| 18:2             | - | linoleic acid  |

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